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ORIGINAL RESEARCH

Detection of Human Papilloma Virus in women in the western region of Libya using Real-time PCR

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ABSTRACT

Purpose: The association between HPV and cervical cancer is well established, however, available data about the detection of the most common high-risk HPV types among Libyan women is still rare. The aim of this study is to detect 24 high-risk HPV types among infected Libyan women in the western region with Pap smear using Real-time PCR.

Materials and Methods: SA total of 50 cervical samples from Pap smears were collected from infected women seeking routine gynecological care and others at Out Patient Department gynecology clinics in the Western Region, National Cancer Institute Sabratha. Real-time PCR was used to facilitate the specific detection of HPV-DNA testing for sequencing genotypes: (16, 18, 26, 30, 31, 33, 34, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 67, 68, 69, 70, 73, 82 and 97).

Results: The overall prevalence of the HPV-DNA high-risk types was 4.0%, and only 2 positive samples (n=2/50) were confirmed of harboring HPV- DNA, while 96% (48/50) had negative HPV detection. The detection of high-risk HPV types was only limited to types 16, 18, and 45, with no detection of the other 21 HPV types.

Conclusion: IThis study showed a low overall prevalence of (4.0%) of HPV infection compared to other regions, there was enough evidence of a high rate burden of HPV 16, 18, and 45 among Libyan women for cervical abnormalities "cancer. *(Open J Biomed Res 2023;2:8-15.)*

Keywords

Cervical cancer, Human papillomavirus, Real-Time PCR, genotyping.

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INTRODUCTION

Human papillomaviruses (HPVs) are small double stranded with a group of more than 150 related viruses. They are divided into low-risk and high-risk varieties according to their propensity to cause cancer. HPVs are the most prevalent sexually transmitted genital infections in the world.[1] Additionally, HPV infection increases the risk of cancer and genital warts in both men and women.[2]

In the early 1980s, Harold Zur Hausen was the first to demonstrate a link between genital HPV infection and cervical cancer. Since then, a strong connection has been discovered between HPV and cervical squamous cell cancer.[3] He was the first to draw attention to the link between HPV infection and cervical cancer, receiving the 2008 Nobel Prize in virology for his pioneering work.[4] According to the International Agency for Research on Cancer (IARC), the human papillomavirus, other viruses, and bacteria have been categorized as carcinogenic to humans under the Monographs program.[1] Furthermore, cervical cancer is the second most prevalent malignancy among women in developing countries and the second major cause of cancer-related deaths.[5] Additionally, it is unclear whether the risk of cancer progression is associated with a

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specific HPV genotype or if it is common for all genotypes. However, it may present for several years, causing cellular changes that, if left untreated, in the genital area (carcinoma of the cervix, vagina, vulva, anal, oral, and penile areas).[2]

The prevalence and distribution of HPV genotypes are influenced by a variety of factors, including patient age, cytology stage, and geographical regions. Moreover, closely related risk factors for progression from cervical infection to cancer include multiple sexual partners, long-term hormonal contraceptive use, co-infection with other pathogens like HIV, Chlamydia trachomatis, and Herpes Simplex type-2, immunosuppression, smoking, and some dietary deficiencies.[1]

Globally, HPV infection may take a long time to lead to progressive cancer, and effects on cancer incidence will take decades to assess.[6] The test is a regular screening of HPV infection for potential malignant conversion that is performed every three years for patients.[7]

Cytology-based screening has been used for several decades as the cytology smear (Pap) for HPV infection; therefore, engaging the screened population is critical to the success of this significant paradigm shift.[6]

Additionally, prophylactic HPV vaccines are given to women with no prior evidence of HPV type-specific infection on a three-dose schedule, a targeted type of HPV infection and high-grade Cervical Intraepithelial Neoplastic (CIN).[8]

MATERIALS AND METHODS

Study population

This cross-sectional descriptive study has been conducted from January to October 2022. Fifty consecutive cervical samples from pap smears were collected from infected women patients seeking routine gynecological care or advice on clinical symptoms such as vaginal discharge or bleeding, menorrhagia, irregular menstruation, genital warts, pelvic pain, and other at OPD gynecology clinics in western regions.

Data and sample collection

The data were collected from the female subjects by specialized gynecologists, including married pregnant and non-pregnant women. All participants were interviewed face to face to collect information about their name, age, date of birth, address, symptoms (complaint), and others, all using a standardized questionnaire. All samples for Pap smear were taken from the transformation zone endocervix by using specific cervical brush (Cytobrush) in a counterclockwise manner, then placed directly in Thin Prep vials (BD Sure Path™) of 96% ethanol transport media. All Liquid-based cervical (LBC) samples were stored at 2c0 to 8 c0 to be preserved.

DNA extraction

The DNA purification was carried out using clinical specimens and an NA extraction kit (BIORON Diagnostics GmbH, Germany).

DNA amplification

The amplification of gene proof was carried out by Real-Time PCR assay in a Rotor-Gene Q system (QIAGEN Hilden, Germany) manufactured in Malaysia. Master Mix and positive control tubes were gently mixed, and then 30 µl of Master Mix was added into PCR tubes. 10 µl of the isolated nucleic acid sample and the positive control was added into individual PCR tubes and mixed well. The total reaction mix volume will be 40 µl. following this step, tubes were tightly closed and then inserted into the device and started amplification according to the manufacturer.

RESULTS

The samples collected from fifty women ranged in age between 18 to 69 years old in the western region of Libya. The age distribution is a method employed by researchers to classify a group of people by their age; it is a quantitative description of how certain population groups divided according to how old they are. However, the mean age of the women under study was (43.4 ± 12.8) years, and according to Fig 1, these ages ranged from 20.0 years to 88.0 years with a median of 41.5 years.

The human papillomavirus test found a positive result for two women (4.0%) and a negative result for forty-eight women (96.0%) among the study population. Furthermore, these two HPV-positive women; they were 44 years and 88 years old, and were residing in the areas of Aleajilat and Altwibia, they complained of recurrent bleeding and bumps in the cervix. Finally, the serotypes of HPV were type 18, and types 16 and 45 respectively.

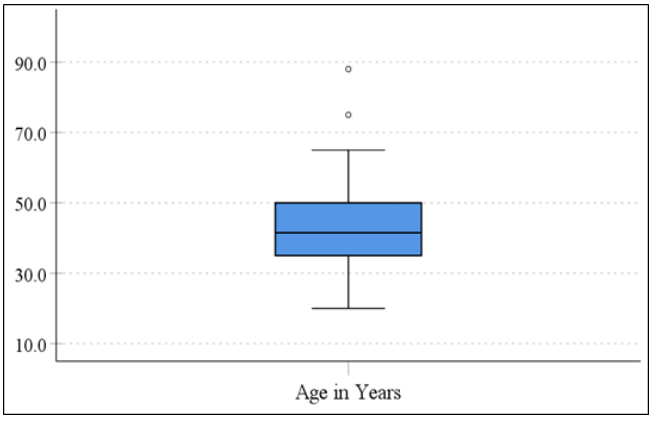

Fig 1. Distribution of women according to the age groups.

Description of curves related to the two positive HPV

Positivity in the FAM channel represents the presence of at least one of 24 high-risk HPV types in the sample. In the case of positivity in other channels except for HEX (Internal control), which allows differentiation of HPV16, 18, and 45, it is possible to confection with other high-risk HPV types as shown in tables 1 and 2, as well as in figures 2-11.

Table 1. Positive women patient in age 44 years.

Channel of curves	Results	Interpretation
Green	Expression	FAM
Yellow	Expression	HEX
Crimson	No expression	HPV 45
Orange	No expression	HPV16
Red	Expression	HPV18

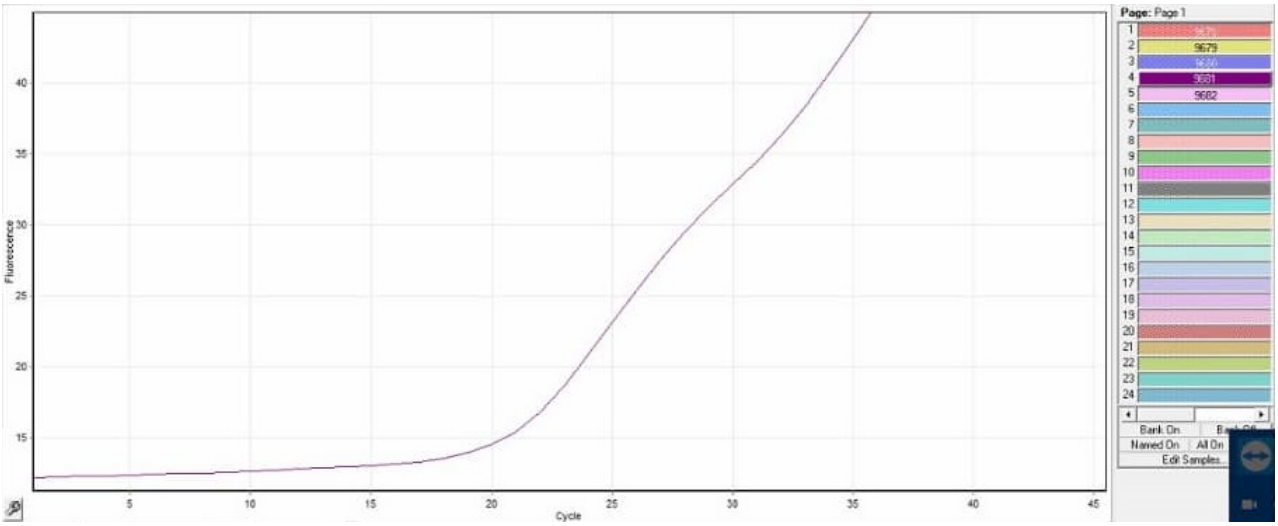

Fig 2. Green fluorescence curve for FAM.

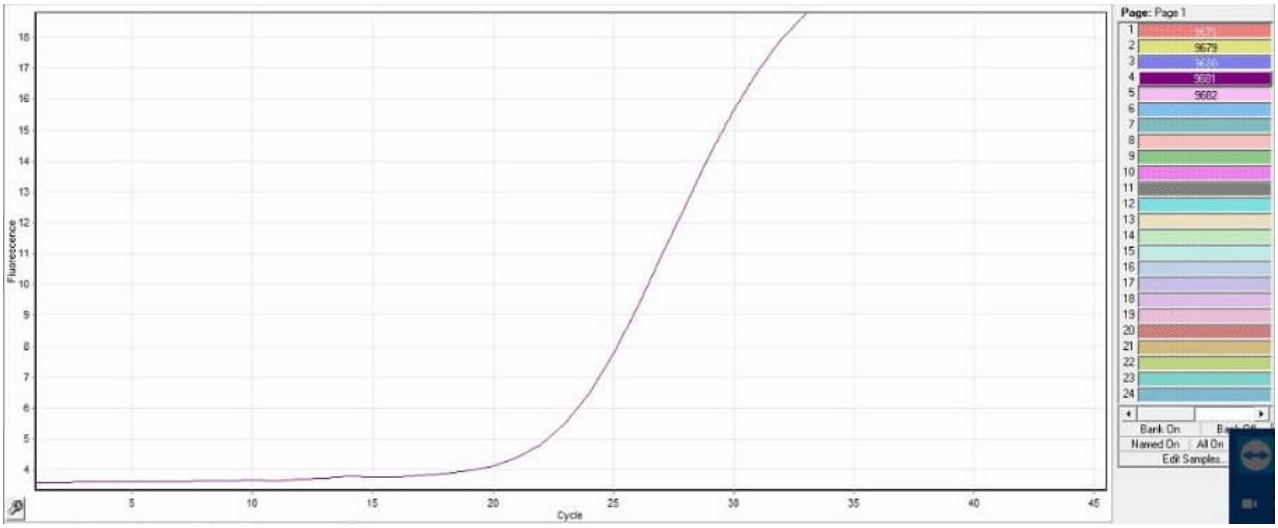

Fig 3. Yellow fluorescence curve for HEX (IC).

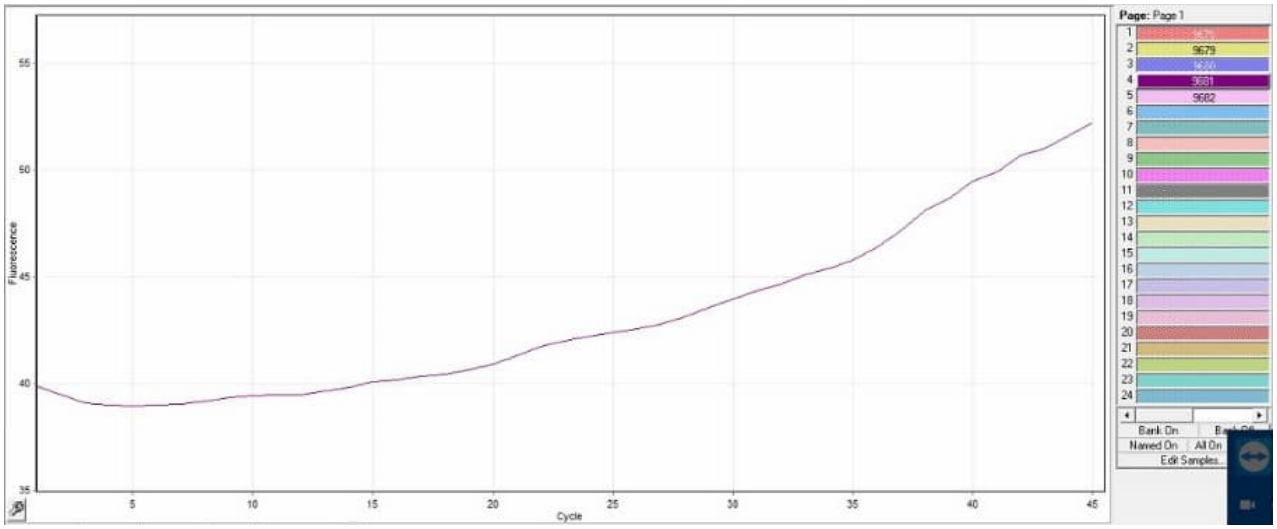

Fig 4. Crimson fluorescence curve for HPV genotypes 45.

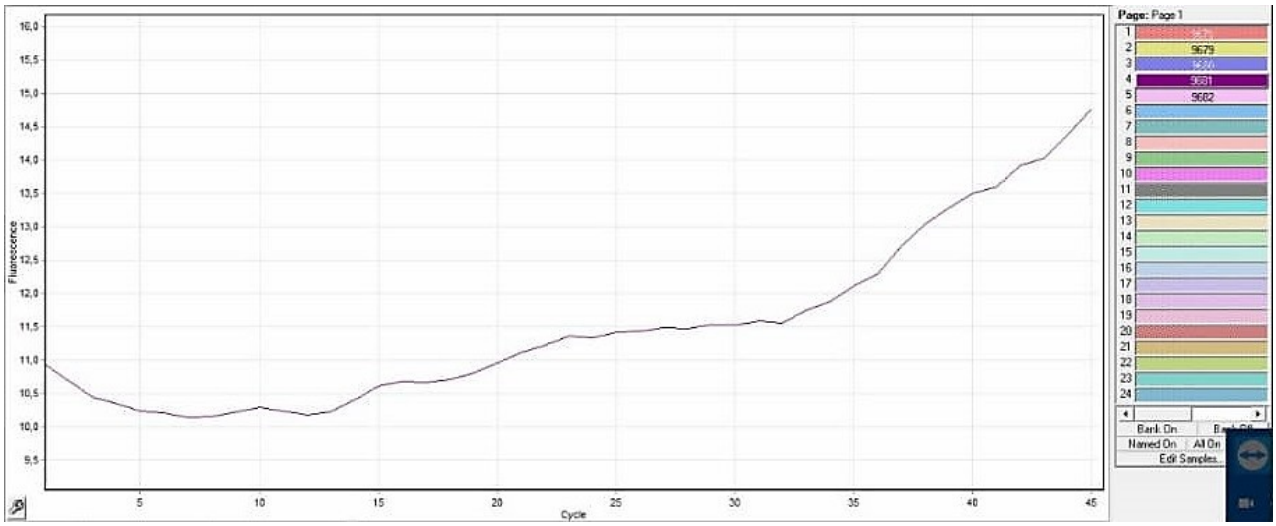

Fig 5. Orange fluorescence curve for HPV genotype 16.

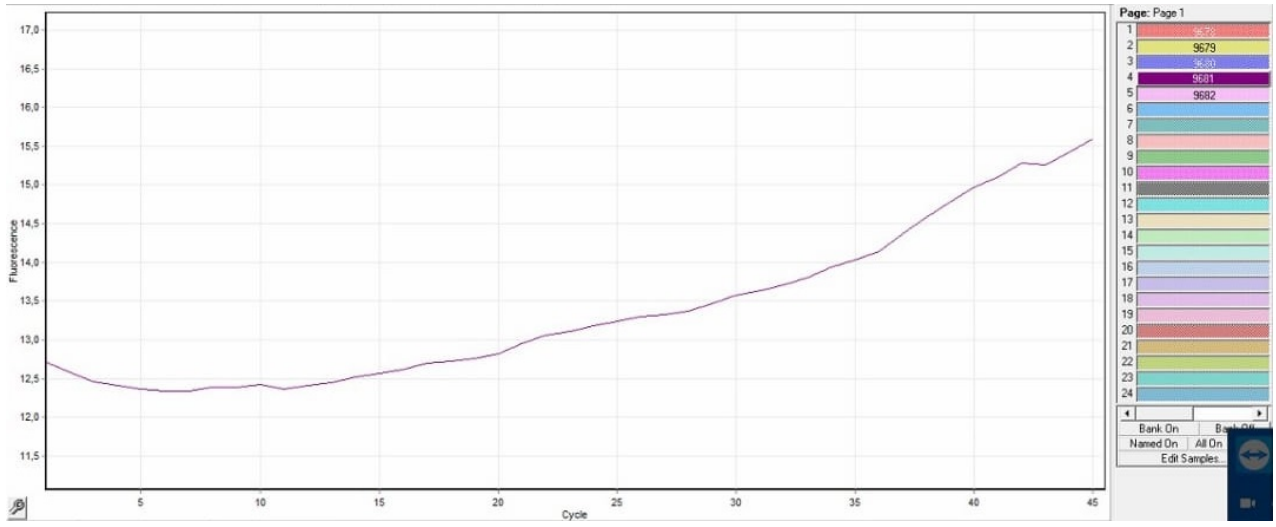

Fig 6. Red fluorescence curve for HPV genotype 18.

Table 2. Positive women patient in age 88 years

Channel of curves	Results	Interpretation
Green	Expression	FAM
Yellow	Expression	HEX
Crimson	Expression	HPV 45
Orange	Expression	HPV16
Red	No expression	HPV18

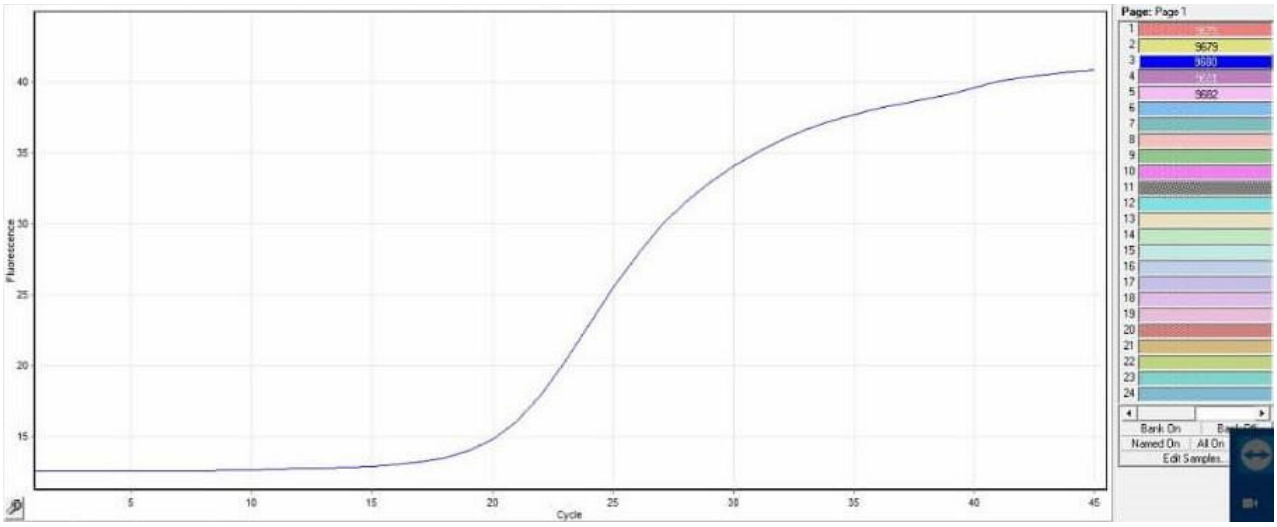

Fig 8. Green fluorescence curve for FAM.

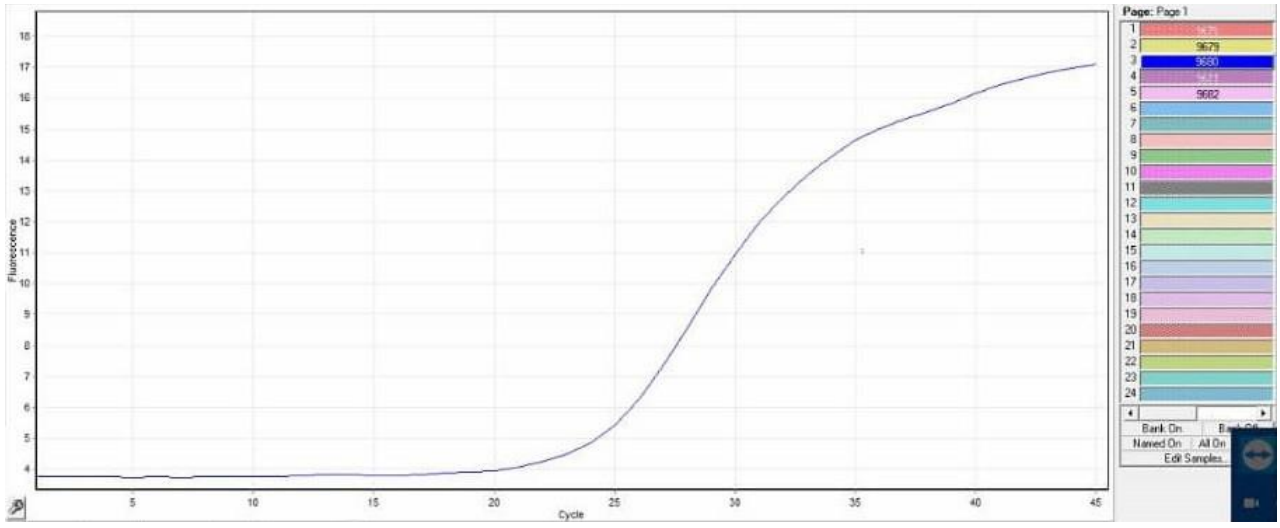

Fig 9. Yellow fluorescence curve for HEX (IC).

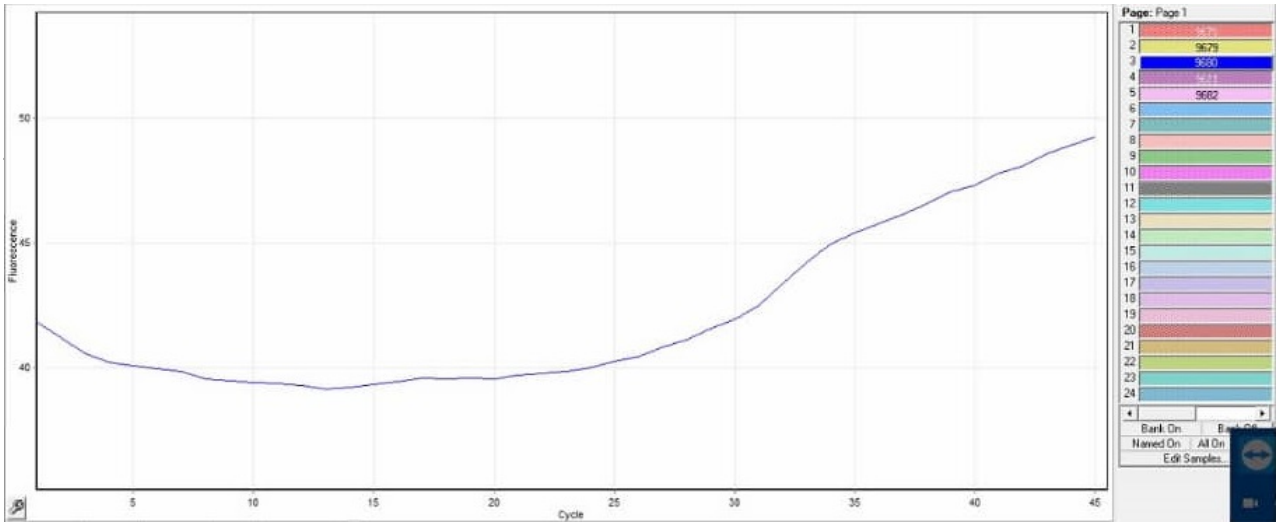

Fig 10. Crimson fluorescence curve for HPV genotypes 45.

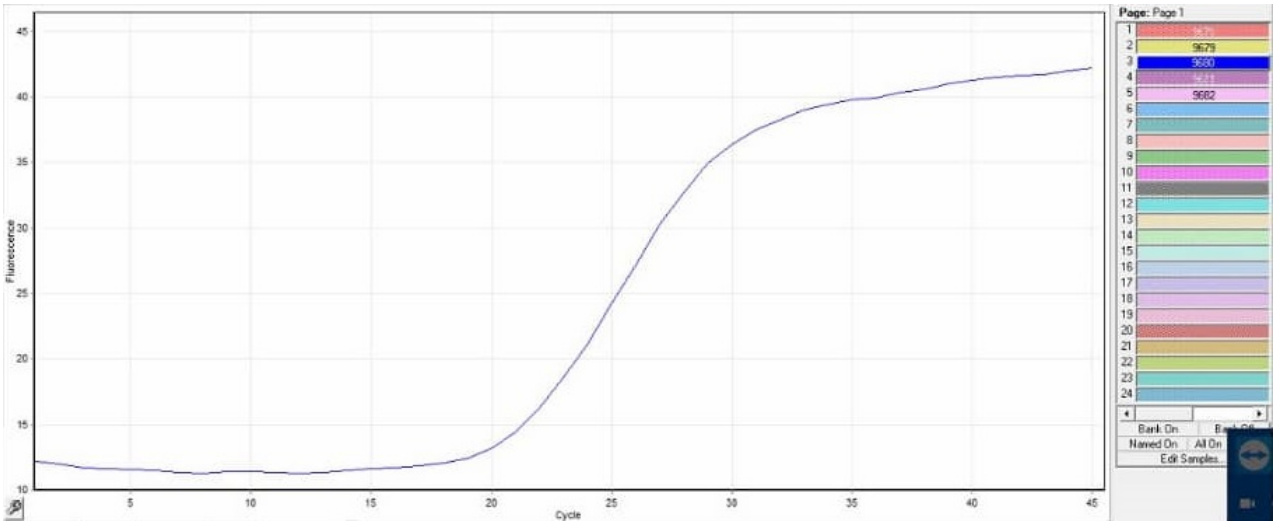

Fig 11. Orange fluorescence curve for HPV genotypes 16 (NO expression).

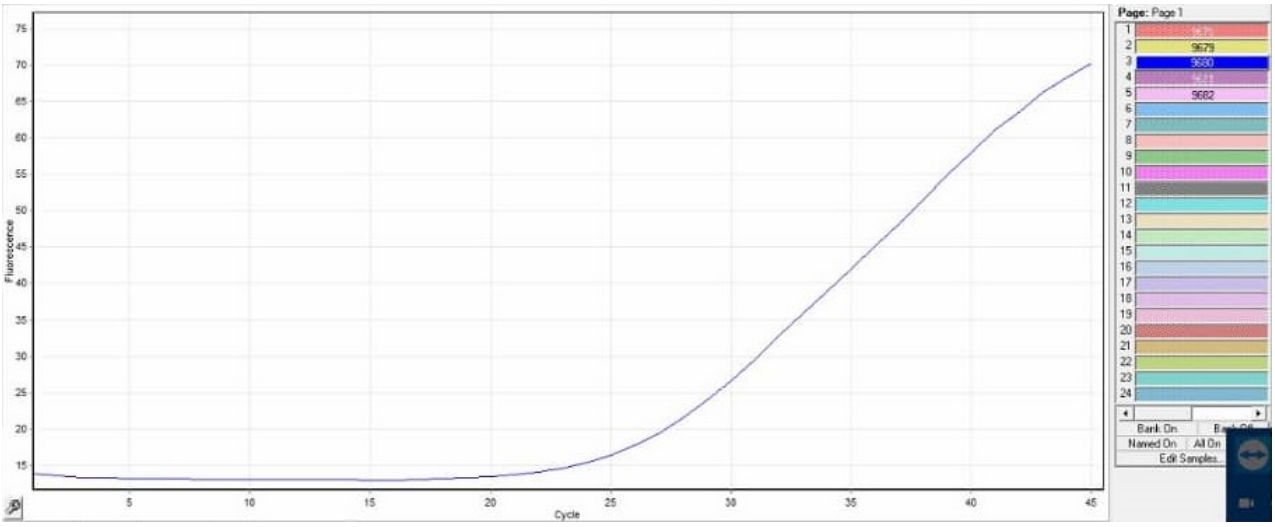

Fig 12. Red fluorescence curve for HPV genotypes 18.

DISCUSSION

This study was performed to investigate the prevalence of the most common 24 high-risk HPV types among infected Libyan women using the powerful molecular technique of Real-time PCR in HPV- DNA detection and genotyping. Although this method of investigation is more sensitive and more specific in the field of virology, the application is not widely used in Libya for the detection of HPV infection. In addition, there was no available data supporting the effectiveness of this technique in the detection of HPV- genotypes isolated from Libyan patients.

On the other hand, a strong association with the current study boasts a significant advantage that collected samples through the liquid-based Pap smear technique, which depended on a clinical examination performed by a consultant gynecologist. This technique facilitated the detection and genotyping of HPV-DNA for molecular diagnosis, which was conducted using Real-time PCR at the Saint James Hospital in Tripoli.

The current study was performed on samples collected from multiple cities in a western region of Libya. It revealed an overall HPV prevalence of 4.0%, which was found to be lower than the prevalence reported in the North Africa region, where the average prevalence of HPV is 21.3%. Although the studies on the prevalence of HPV in this region are not abundant.[1]

The relative detection of HPV among women with cervical abnormalities found in Libya (4.5%) in a study performed by Alzaquzi et al[9] is consistent with the incidence of cervical cancer in a study performed in Qatar by Elmi et al,[7] which reported that HPV infection is the third leading cause of female cancer.

In a more detailed comparison to neighboring countries, the current study was found to be higher than in Algeria (3.6%),[10] whereas Ardhaoui et al., reported in 2016 that the HPV prevalence was in Tunisia (13.2%),[11] Morocco (15.7%),[12] and as high as (40.8%) in Egypt.[13]

Remarkably, the current study showed that the prevalence of HPV infection in agreement with the previous study documented in Libya,[9] that HPV16 is the most high-risk type distributed in Libya as well as it is the most common high-risk type of HPV distributed worldwide.

The specific HPV genotyping showed a predominant detection of double infections that were positive for HPV-DNA in the current study that was found in only one case (HPV 16 and 45). Double infections were also detected in two cases (HPV 16 and 18) with a previous study performed in morocco.[12] The current results are in agreement with previous study in the incidence of double HPV infection in one case sample but with a different in HPV genotypes.

Remarkably, the current study of HPV genotyping showed detection of the most common HPV types (16, 18) which has an agreement with the study performed in Iran by Mortazavi SH et al.[14] Whilst, current results showed a wide disparity in HPV genotyping among positive cases of HPV (16, 18, and 45), a previous study performed in Libya showed that HPV 16 was the solely detected HPV genotype.[9]

The current study showed only a small number of HPV-positive cases with a peak of infection in age groups ranging between 35 and 45 years with an average age of 41.2%. In contrast, it globally reported that young women less than 35 years old revealed the highest infection rate following their first sexual activity.[15] This could be attributed to the restriction of multiple sexual partners as an important major risk factor for HPV infection among Libyan girls during their early reproductive life.

Notably, all positive cases were collected from women who presented to a gynecology clinic seeking advice on clinical symptoms. This suggests a lack of awareness towards routine Pap smear testing as an efficient tool for early detection of cervical epithelial changes to avoid cervical cancer.

It is worth noting here that the collection of samples was only from married women attending outpatient gynecology clinics due to cultural issues. This could lead to selection bias, and maybe the women's general population is not represented in the current study; this would be a limitation.

CONCLUSION

Although the current study showed a low overall prevalence of (4.0%) of HPV infection compared to other regions, there was enough evidence of a high rate burden of HPV 16, 18, and 45 among Libyan women for cervical abnormalities "cancer". The introduction of HPV-DNA testing combined with a liquid-based Pap smear test would greatly benefit cervical cancer screening and management. Early detection of HPV infection using real-time PCR would help prevent cervical lesions progression to devastating cervical cancer. In addition, it could greatly aid the medical authorities in assessing HPV vaccination's impact on the targeted population.

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